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(54) Procedure for obtaining the somatostatin analog, octreotide

Verfahren zur Herstellung des Somatostatin Analogons Octreotide

Procédé de préparation de l'Octreotide, un analogue de la somatostatin

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- **WU Y -T ET AL: "Facile solid phase synthesis of octreotide analogs using p -carboxybenzaldehyde as a novel linker to anchor Fmoc-threoninol to solid phase resins" TETRAHEDRON LETTERS, vol. 39, no. 13, 26 March 1998, page 1783-1784 XP004108476**
- **W. B. EDWARDS ET AL: "Generally applicable convenient solid-phase synthesis and receptor affinities of ocreotide analogs" J. MED. CHEM., vol. 37, 1994, pages 3749-3757, XP002102596**
- **ARANO Y. ET AL: 'Conventional and high-yield synthesis of DTPA-conjugated peptides: Application of a monoreactive DTPA to DTPA-D-Phe-1-octreotide synthesis' BIOCONJUGATE CHEMISTRY vol. 8, 1997, pages 442 - 446**

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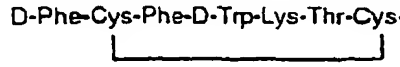
Description

Field of the Invention

[0001] This invention involves a procedure for preparation of the somatostatin analog, octreotide and its pharmaceutically acceptable salts formed by addition of acids or complexes of the same. Likewise, the invention is related to the preparation of intermediate compounds useful in the synthesis of octreotide in accordance with the invention.

Basis of the Invention

[0002] While somatostatin possesses a very broad therapeutic potential and could be administered in a wide variety of clinical applications, its mean half-life in plasma is extremely short, reducing the number of applications possible. This drawback has prompted a number of research groups to establish the goal of developing more stable and more powerful analogs of somatostatin. One of these groups made a number of tests with cyclic octapeptides. One of these octapeptides yielded excellent biological activity both in vitro and in vivo **(Pless J., Metabolism, 41, 5-6, (1992)).** This analog is Octreotide. Its structure is shown below:



[0003] The presence of a D-phenylalanine in the N-terminal end and an amino alcohol in the C-terminal end, along with the D-tryptophan residue and the disulfide bridge, make the molecule very resistant to metabolic degradation. The octreotide permits a 24-hr. incubation in aggressive medium such as gastric juices or in intestinal mucosa.

[0004] Octreotide inhibits growth hormone for a lengthy period, inhibits the secretion of glucagon to a lesser degree, and inhibits insulin secretion only in a transient manner. It is thus more selective than other somatostatin analogues in regulating the levels of growth hormone in the body and therefore at present is indicated in acromegaly to control and reduce the plasma levels of such hormone. It is also used in the treatment of cellular alterations of gastroenteropancreatic endocrine origin and of certain types of tumors.

State of the Art

[0005] The primary ocreotide preparation described is a classic synthesis in solution **(Bauer W., Pless J., (Sandoz) Eur. Pat. Appl. 29,579. Eldem U.S. Pat. 4,395,403 (1981, 1983).** Syntheses in solid phase have been described subsequently **(Mergler et al, Alsina et al, Neugebauer).** In all of them, the objective is to form the entire peptide chain by solid phase peptide synthesis, starting the synthesis by the threoninol residue. This makes it mandatory to protect this residue.

[0006] The first author **(Mergler M., Hellstern H., Wirth W., Langer W., Gysi P. and Prikoszovich W., Peptides: Chemistry and Biology. Proceedings of the 12th American Peptide Symposium. Smith, J.A. and Rivier J.E. Eds ESCOM, Leiden, Poster 292 Presentation, (1991).)** describes a synthetic process, using an aminomethyl resin upon which the Threoninol residue is incorporated with the two alcohol functions protected in acetal form. They carry out the synthesis following an Fmoc/tBu protection scheme, forming the disulfide bridge on resin by oxidation of the thiol groups of the previously deprotected cysteine residues and releasing and deprotecting the peptide with a 20% mixture of TFA/DCM.

[0007] In early 1997, **Alsina J.** et al. **(Alsina J., Chiva C., Ortiz M., Rabanal F., Giralt E. and Albericio F., Tetrahedron Letters, 38, 883-886, (1997))** described the incorporation, on active carbonate resins, of a Threoninol residue with the amino group protected by the Boc group and the side chain protected by a Bzl group. The synthesis was then continued by Boc/Bzl strategy. Formation of the disulfide bridge was carried out directly on resin using iodine, and the peptide was cleaved from the resin and it's side chain protecting groups were simultaneously removed with HF/anisole 9/1. At a final stage the formyl group was removed with a piperidine/DMF solution. Neugebauer **(Neugebauer W., Lefevre M.R., Laprise R, Escher E., Peptides: Chemistry, Structure and Biology, p 1017, Marshal G.R. and Rivier J.E. Eds. ESCOM. Leiden (1990))** described a linear synthesis with a yield of only 7%.

[0008] Edwards et al. (Edwards B.W., Fields C.G., Anderson C.J., Pajeau T.S., Welch M.J., Fields G.B., J. Med. Chem. 37 3749-3757 (1994)) carried out another solid-phase type approximation; they synthesized step-by-step on the resin, the peptide D-Phe-Cys(Acm)-Phe-D-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys(Acm)-HMP-resin. Next, they proceeded to form the disulfide on resin and then released the peptide from the resin by means of aminolysis with threoninol, with obtaining a total yield of only 14%.